
PHYLOGENETIC RELATIONSHIPS IN THE TRIBES ANCHONIEAE, CHORISPOREAE, EUCLIDIEAE, AND HESPERIDEAE (BRASSICACEAE) BASED ON NUCLEAR RIBOSOMAL ITS DNA SEQUENCES

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ABSTRACT

Sequence data from the nuclear ITS region of 118 species (152 accessions) were used to test the monophyletic status and interrelationships of four related tribes in the Brassicaceae: Anchonieae, Chorisporeae, Euclidieae, and Hesperideae. Both maximum parsimony and maximum likelihood analyses support the recognition of the tribes Hesperideae (unigeneric, *Hesperis* L.) and Chorisporeae (3 genera, including *Chorisporea* R. Br. ex DC., *Diptychocarpus* Trautv., and *Parrya* R. Br.), whereas the Anchonieae and the Euclidieae each separate into two distinct and distant clades (designated here as Anchonieae I and II and Euclidieae I and II). The data also support the exclusion of eight genera from the latter four tribes, with appropriate tribal assignment given in parentheses: *Aubrieta* Adans. (Arabideae), *Blennodia* R. Br. (Camelineae), *Erysimum* L. (Camelineae), *Goldbachia* DC. (unresolved), *Hesperidanthus* (B. L. Rob.) Rydb. (Schizopetaleae), *Notothlaspi* Hook. f. (unresolved), *Pseudocamelina* (Boiss.) N. Busch (Thlaspidaceae), and *Zuranda* (Dvorák) Askeroova (unresolved). The genus *Malcolmia* R. Br. (ca. 35 species) was paraphyletic and divided into three tribal clades, providing support for the separate genera *Malcolmia*, *Strigosella* Boiss., and *Zuranda*. The genera *Anchonium* DC., *Desideria* Pamp., *Eremobium* Boiss., *Erysimum*, *Iskandera* N. Busch, *Matthiola* R. Br., *Morettia* DC., *Neotorularia* Hedge & J. Léonard, *Rhammatophyllum* O. E. Schulz, *Sisymbriopsis* Botsch. & Tzvelev, *Sterigmotemum* M. Bieb., and *Tetracme* Bunge each fell within a single tribal clade but were not monophyletic.

Key words: Anchonieae, Chorisporeae, Euclidieae, Hesperideae, ITS, Brassicaceae.

The Brassicaceae are a large family of ca. 338 genera and over 3700 species distributed throughout the world, primarily in temperate regions (Al-Shehbaz, 1984; Al-Shehbaz et al., 2006; Warwick & Al-Shehbaz, 2006). They are a natural family easily distinguished by floral and fruit morphology (e.g., cruciform corolla, tetradynamous stamens, and characteristic siliques). Tribal classification of the family is, however, problematic and not well understood phylogenetically. The characters traditionally used at this rank are few and include orientation of the radicle in the embryo, number of rows of seed in each locule, flower color, trichome type, and features of the nectaries. Numerous studies have shown these morphological characters to be highly homoplasious and therefore not phylogenetically informative on a family-wide basis (reviewed in Al-Shehbaz et al., 2006). Several tribal systems have been proposed and, with the exception of the Brassicaceae, few, if any, of the other traditional tribes are monophyletic (Hedge,

1976; Al-Shehbaz, 1984; Koch et al., 2001; Al-Shehbaz et al., 2006).

The first comprehensive molecular-based overviews on the phylogeny of the family are now emerging (Bailey et al., 2006; Beilstein et al., 2006). The present paper tests the monophyletic status and interrelationships of four closely related tribes in the Brassicaceae: the Anchonieae DC. (12 genera), Chorisporeae Ledeb. (2 genera), Euclidieae DC. (25 genera), and Hesperideae Prantl (1 genus), as recently redefined by Al-Shehbaz et al. (2006). In Beilstein et al. (2006), these tribes corresponded to four separate, strongly supported *ndhF*-based clades, designated Q, S, P, and R, respectively, within one of three well-supported lineages, designated III in figure 2 of their study. Sampling from these tribes, however, was limited in that study, with sequence data from only 4, 2, 12, and 2 taxa, respectively. Similarly, few sequences were available for these tribes in the ITS-based study of Bailey et al. (2006), i.e. Anchonieae (2

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Table 1. Comparison of traits in the four related tribes Anchonieae, Chorisporeae, Euclidieae, and Hesperideae, modified from Al-Shehbaz et al. (2006).

Trait	Anchonieae	Chorisporeae	Euclidieae	Hesperideae
Trichomes	branched	simple, not branched	branched and simple	simple and/or forked
Glands	multicellular	multicellular	glands absent	unicellular
Stalk of glands	multiseriate	multiseriate	glands absent	uniseriate
Siliques	dehiscent, not splitting into 1-seeded segments	moniliform fruits, 1-seeded segments	dehiscent or tardily so, no 1-seeded segments	tardily dehiscent
Stigma lobes	strongly 2-lobed	connivent	entire or 2-lobed	decurrent
Sepals	erect	erect, closed calyx	erect	erect
Cotyledons	accumbent or incumbent	accumbent	incumbent	incumbent
Base chromosome number	$x = 7$	$x = 7$	$x = 7$	$x = 6-10$
Number of genera	12	2	25	1
Number of species	130	12	150	46

genera), Hesperideae (1 species), Euclidieae (3 genera); the three tribes formed a loosely supported single clade, with greater support for the Euclidieae (70% bootstrap support). These tribes also formed a clade in recent *trnF*-based sequence studies of Koch et al. (2006), with further support for their relatedness suggested by the shared presence of a large insertion in the *trnL* intron. Similarly, in *matK* and *Chs*-based sequences studies, five genera assigned to these tribes formed a well-supported monophyletic group (Yue et al., 2006).

All genera placed in the tribes Anchonieae, Chorisporeae, and Hesperideae have glands, whereas members of the Euclidieae do not (Table 1; Al-Shehbaz et al., 2006). These glands are multicellular with multiseriate stalks in the Anchonieae and Chorisporeae, but unicellular on uniseriate stalks in the Hesperideae. The primary difference between the Anchonieae and Chorisporeae is the presence of 1-seeded fruit segments in the latter tribe. Genera in the four tribes have been subjected to various tribal placements in the past (see Table 2). Schulz (1936) placed the genera with multicellular glands, along with a highly heterogeneous assemblage of other genera, in the tribes Hesperideae and Matthioleae O. E. Schulz, distinguishing the two solely on the basis of cotyledonary position, and recognized the Euclidieae as a separate tribe. These three tribes were later combined by Janchen (1942) as the Hesperideae, subtribes Hesperidinae, Matthiolinae, and Euclidiinae, respectively. Al-Shehbaz (1984) recognized a single large tribe, the Hesperideae, which included the Anchonieae, Cheirantheae Webb & Berth., Erysimeae Dumort., Matthioleae, and Schizopetaleae R. Br. ex Barn. Al-Shehbaz (1988) later combined the Hesperideae and Matthioleae under the earlier name Anchonieae (ca. 27 genera with multicellular glands

and/or connivent decurrent stigmatic lobes). Although *Erysimum* L. was retained in the Anchonieae in his 1988 treatment, Al-Shehbaz noted that careful evaluation of its tribal position was needed.

The Hesperideae, as redefined by Al-Shehbaz et al. (2006), are unigeneric (*Hesperis* L.) and distinguished from the rest of the family by having glands with uniseriate stalks terminated with a unicellular gland. Unicellular glands are also found in *Descurainia* Webb & Berthel., but they are unstalked and apparently not homologous to those of the Hesperideae or the multicellular glands of the tribes Anchonieae and Chorisporeae (Al-Shehbaz, 1988; Al-Shehbaz et al., 2006). The absence of multicellular glands is evident among some members of the Anchonieae and is probably a derived state (Al-Shehbaz, 1988; Jacquemoud, 1988). The genera *Dontostemon* Andr. ex C. A. Mey., *Hesperis*, *Matthiola* R. Br., and *Parrya* R. Br. include species with and without multicellular glands, while *M. longipetala* (Vent.) DC. and *P. nudicaulis* (L.) Regel often have glandular or eglandular plants within the same population.

The Anchonieae, as defined by Al-Shehbaz et al. (2006), include 12 genera and about 130 species (see Table 2). The *ndhF*-based phylogenetic study of Beilstein et al. (2006) revealed a well-supported Anchonieae clade that included *Matthiola* (2 species), *Oreoloma* Botsch. (1 species), and *Sterigmostemum* M. Bieb. (1 species). The tribe is characterized by the presence of multicellular-multiseriate glands (rarely eglandular), branched trichomes, often strongly 2-lobed stigmas, and erect sepals (Table 1). The tribe is distributed primarily in Eurasia and northern and tropical Africa, with only four species of *Parrya* extending into North America. Dvorák (1972) considered Pacific North America and northeastern Asia as

Table 2. Taxonomic tribal assignment of genera in the Anchonieae, Chorisporeae, Hesperideae, and Euclidieae. Species numbers from Warwick et al. (2006a), and chromosome numbers from Warwick and Al-Shehbaz (2006). Tribal assignments in the present study in conflict with Al-Shehbaz et al. (2006) are indicated in bold. n = haploid chromosome number; $2n$ = diploid chromosome number.

Genus	Schulz (1936)	Janchen (1942)	Al-Shehbaz (1988)	Al-Shehbaz et al. (2006)	Present study	No. of species	Base chromosome no. (x)	Chromosome no. (n ; $2n$)
<i>Anastatica</i> L.	Euclidieae	Hesperideae-Euclidiinae	—	Euclidieae	—	1	$x = 11$	$2n = 22$
<i>Anchonium</i> DC.	Hesperideae	Hesperideae-Hesperidinae	Anchonieae	Anchonieae	Anchonieae I	2	$x = 7, 8$	$n = 7$; $2n = 14, 16, 21$
<i>Atelantha</i> Hook. f. & Thomson	Hesperideae	Hesperideae-Hesperidinae	—	Euclidieae	—	1	—	—
<i>Aubrieta</i> Adans.	Matthioleae	Arabideae-Arabinae	—	Arabideae	Arabideae	12	$x = 8$	$n = 8$; $2n = 16, 32$
<i>Blennodia</i> R. Br.	Hesperideae	Hesperideae-Hesperidinae	—	—	Camelineae	2	$x = 12$	$n = 12$
<i>Boreava</i> Jaub. & Spach	Euclidieae	Sisymbrieae-Buniadinae	—	Isatideae	—	2	$x = 7$	$2n = 14$
<i>Braya</i> Sternb. & Hoppe	Sisymbrieae-Brayinae	Sisymbrieae-Brayinae	—	Euclidieae	Euclidieae I	13	$x = 14, 16$	$n = 14, 16, 21, 28, 32, 35$; $2n = 28, 32, 42, 48, 56, 64, 70$
<i>Bunias</i> L.	Euclidieae	Sisymbrieae-Buniadinae	Anchonieae	Anchonieae	—	3	$x = 7$	$n = 7$; $2n = 14, 28, 42$
<i>Cheiranthus</i> L. [= <i>Erysimum</i> L.]	Hesperideae	Hesperideae-Matthiolinae	—	Camelineae, <i>Cheiranthus</i> [= <i>Erysimum</i>]	Camelineae			
<i>Chorispora</i> R. Br. ex DC.	Matthioleae	Hesperideae-Matthiolinae	Anchonieae	Chorisporeae	Chorisporeae	11	$x = 7, 9$	$n = 7, 9, 14$; $2n = 14$
<i>Cithareloma</i> Bunge	Matthioleae	Hesperideae-Matthiolinae	—	Euclidieae	Euclidieae II	3	$x = 13$	$n = 13$; $2n = 26$
<i>Clausia</i> Korn.-Trotzky	Hesperideae, <i>Clausia</i> [= <i>Hesperis</i> L.]	Hesperideae-Matthiolinae	Anchonieae	Anchonieae	Anchonieae II	6	$x = 7$	$2n = 14, 28$
<i>Cryptospora</i> Kar. & Kir.	Hesperideae	Hesperideae-Hesperidinae	—	Euclidieae	Euclidieae I	3	$x = 7$	$2n = 14$
<i>Desideria</i> Pamp.	Arabideae, <i>Desideria</i> [= <i>Ermania</i> Cham.]	—	—	Euclidieae	Euclidieae I	12	$x = 7$	$2n = 14$
<i>Diceratella</i> Boiss.	Matthioleae	Hesperideae-Matthiolinae	Anchonieae	Euclidieae	Euclidieae II	11	$x = 11$	$n = 11$

Table 2. Continued.

Genus	Schulz (1936)	Janchen (1942)	Al-Shehbaz (1988)	Al-Shehbaz et al. (2006)	Present study	No. of species	Base chromosome no. (x)	Chromosome no. (n; 2n)
<i>Dichasianthus</i> Ovcz. & Junussov	—	—	—	Euclidieae	Euclidieae I	1	—	—
<i>Dilophia</i> Thomson	Lepidieae-Cochleariinae	—	—	Euclidieae	—	2	—	—
<i>Diptychocarpus</i> Trautv.	Matthioleae	Hesperideae-Matthiolinae	Anchonieae	Chorisporeae	Chorisporeae	1	x = 7	n = 7; 2n = 14
<i>Dontostemon</i> Andrz. ex C. A. Mey.	Arabideae	—	Anchonieae	Anchonieae	Anchonieae II	11	x = 7	n = 7; 2n = 14, 28
<i>Eremobium</i> Boiss.	Hesperideae	Hesperideae-Hesperidinae	—	Euclidieae	Euclidieae II	2	x = 8, 10, 13	n = 10, 13; 2n = 16, 20
<i>Erysimum</i> L.	Hesperideae	Hesperideae-Hesperidinae	Anchonieae	Camelineae	Camelineae	223	x = 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17	n = 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 21, 24, 27, 28; 2n = 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 48, 54, 56, 72
<i>Euclidium</i> R. Br.	Euclidieae	Hesperideae-Euclidiinae	—	Euclidieae	Euclidieae I	1	x = 7	n = 7; 2n = 14
<i>Goldbachia</i> DC.	Hesperideae	Sisymbrieae-Buniadinae	—	—	Unresolved	5	x = 14	n = 14; 2n = 14, 28
<i>Hesperidanthus</i> (B. L. Rob.) Rydb.	Matthioleae	Hesperideae-Matthiolinae	—	Schizopetaleae	Schizopetaleae	5	x = 11	n = 11, 20
<i>Hesperis</i> L.	Hesperideae	Hesperideae-Hesperidinae	Anchonieae	Hesperideae	Hesperideae	46	x = 6, 7, 8, 10	n = 6, 7, 8, 12, 14, 16, 20, 24; 2n = 12, 14, 16, 24, 28
<i>Hymenophysa</i> C. A. Mey. [= <i>Lepidium</i> L.]	Euclidieae	Lepidieae-Lepidiinae, <i>Hymenophysa</i> [= <i>Cardaria</i> Desv.]	Lepidieae	Lepidieae, <i>Hymenophysa</i> [= <i>Lepidium</i>]	—	—	—	—
<i>Iodanthus</i> Torr. & A. Gray ex Steud.	Matthioleae	Hesperideae-Matthiolinae	Arabideae	Cardamineae	—	1	—	—
<i>Iskandera</i> N. Busch	—	—	Anchonieae	Anchonieae	Anchonieae I	2	—	—
<i>Lachnoloma</i> Bunge	Euclidieae	Hesperideae-Euclidiinae	—	—	—	1	x = 7	2n = 14

Table 2. Continued.

Genus	Schulz (1936)	Janchen (1942)	Al-Shehbaz (1988)	Al-Shehbaz et al. (2006)	Present study	No. of species	Base chromosome no. (x)	Chromosome no. (n; 2n)
<i>Leiospora</i> (C. A. Mey.) F. Dvorák	—	—	—	Euclidieae	Euclidieae I	6	$x = 7$	$2n = 14$
<i>Leptaleum</i> DC.	Hesperideae	Hesperideae-Hesperidinae	—	Euclidieae	Euclidieae I	1	$x = 7$	$n = 7$; $2n = 14$
<i>Litwinowia</i> Woronow	Euclidieae, <i>Litwinowia</i> [= <i>Euclidium</i>]	Hesperideae-Euclidiinae	Anchonieae	—	—	1	$x = 7$	$n = 7$; $2n = 14$
<i>Lonchophora</i> Durieu [= <i>Matthiola</i> R. Br.]	Matthioleae	Hesperideae-Matthiolinae	Anchonieae	Anchonieae	Anchonieae I	—	—	—
<i>Malcolmia</i> R. Br.	Hesperideae	Hesperideae-Hesperidinae	Anchonieae	Euclidieae	Euclidieae I and II; Camelinaeae	32	$x = 7, 8, 10$	$n = 7, 8, 10, 14, 16$; $2n = 14, 16, 20, 24, 28, 32$
<i>Maresia</i> Pomel	Hesperideae	Hesperideae-Hesperidinae	—	Euclidieae	Euclidieae II	3	$x = 14$	$2n = 28$
<i>Mathewsia</i> Hook. & Arn.	Hesperideae	Hesperideae-Hesperidinae	—	Schizopetaleae	Unresolved	10	—	—
<i>Matthiola</i> R. Br.	Matthioleae	Hesperideae-Matthiolinae	Anchonieae	Anchonieae	Anchonieae I	48	$x = 5, 6, 7, 8, 13$	$n = 6, 7, 8, 13$; $2n = 10, 11, 12, 14, 16, 24, 28$
<i>Microstigma</i> Trautv.	Matthioleae, <i>Microstigma</i> [= <i>Matthiola</i>]	Hesperideae-Matthiolinae	Anchonieae	Anchonieae	Anchonieae I	3	$x = 6$	$2n = 12$
<i>Morettia</i> DC.	Matthioleae	Hesperideae-Matthiolinae	Anchonieae	Euclidieae	Euclidieae II	3	$x = 11$	$n = 11$
<i>Myagrum</i> L.	Euclidieae	Sisymbrieae-Isatidinae	—	Isatideae	—	1	$x = 7$	$n = 7$; $2n = 14$
<i>Neotorularia</i> Hedge & J. Léonard	Sisymbrieae-Brayinae	Hesperideae-Hesperidinae	—	Euclidieae	Euclidieae I	11	$x = 7$	$n = 7$; $2n = 14, 28, 42$
<i>Neslia</i> Desv.	Euclidieae	Lepidieae-Capsellinae	—	Camelinaeae	Camelinaeae	1	$x = 7$	$n = 7, 14, 21$; $2n = 14, 42$
<i>Votoceras</i> R. Br.	Matthioleae	Hesperideae-Matthiolinae	—	Euclidieae	Euclidieae II	1	$x = 11$	$n = 11$; $2n = 22$
<i>Notothlaspi</i> Hook. f.	Lepidieae-Notothlaspidinae	Lepidieae-Notothlaspidinae	—	Euclidieae	Notothlaspi clade	2	$x = 19$	$n = 19$
<i>Ochthodium</i> DC.	Euclidieae	Sisymbrieae-Buniadinae	—	—	—	1	—	—

Table 2. Continued.

Genus	Schulz (1936)	Janchen (1942)	Al-Shehbaz (1988)	Al-Shehbaz et al. (2006)	Present study	No. of species	Base chromosome no. (<i>x</i>)	Chromosome no. (<i>n</i> ; <i>2n</i>)
<i>Octoceras</i> Bunge	Euclidieae	Hesperideae-Euclidiinae	—	—	—	1	<i>x</i> = 7	<i>2n</i> = 14
<i>Oreoloma</i> Botsch.	—	—	Anchonieae	Anchonieae	Anchonieae I	3	—	—
<i>Parolinia</i> Webb	Matthioleae	Hesperideae-Matthiolinae	Anchonieae	Euclidieae	Euclidieae II	5	<i>x</i> = 11	<i>2n</i> = 22
<i>Parrya</i> R. Br.	Matthioleae	Hesperideae-Matthiolinae	Anchonieae	Anchonieae	Chorisporeae	34	<i>x</i> = 7	<i>2n</i> = 14, 21, 28
<i>Pseudocamelina</i> (Boiss.) N. Busch	Matthioleae	Lepidieae-Cochleariinae	—	Thlaspideae	Thlaspideae	3	<i>x</i> = 7	<i>n</i> = 7; <i>2n</i> = 14
<i>Pseudoclausia</i> Popov	—	—	Anchonieae	Anchonieae	—	10	<i>x</i> = 7	<i>n</i> = 7; <i>2n</i> = 14
<i>Pycnoplithus</i> O. E. Schulz	Hesperideae	Hesperideae-Hesperidinae	—	—	—	1	—	—
<i>Rhammatophyllum</i> O. E. Schulz	Hesperideae	Hesperideae-Hesperidinae	—	Euclidieae	Euclidieae I	10	<i>x</i> = 8	<i>2n</i> = 16
<i>Schimpera</i> Hochst. & Steud. ex Endl.	Euclidieae	Sisymbrieae-Buniadinae	—	Isatideae	—	1	<i>x</i> = 7	<i>n</i> = 7; <i>2n</i> = 14
<i>Shangrilaia</i> Al-Shehbaz, J. P. Yue & H. Sun	—	—	—	Euclidieae	—	1	—	—
<i>Sisymbriopsis</i> Botsch. & Tzvelev	—	—	—	Euclidieae	Euclidieae I	5	—	—
<i>Solms-laubachia</i> Muschl.	Matthioleae	Hesperideae-Matthiolinae	—	Euclidieae	Euclidieae I	9	<i>x</i> = 7	<i>2n</i> = 14, 28
<i>Spirorhynchus</i> Kar. & Kir.	Euclidieae	Sisymbrieae-Buniadinae	—	Isatideae	—	1	<i>x</i> = 7	<i>2n</i> = 14
<i>Sterigmostemum</i> M. Bieb.	Hesperideae	Hesperideae-Hesperidinae	Anchonieae	Anchonieae	Anchonieae I	7	<i>x</i> = 7	<i>n</i> = 7; <i>2n</i> = 14
<i>Strigosella</i> Boiss.	Hesperideae, <i>Strigosella</i> [= <i>Malcolmia</i>]	Hesperideae-Hesperidinae	Anchonieae, <i>Strigosella</i> [= <i>Malcolmia</i>]	Euclidieae, <i>Strigosella</i> [= <i>Malcolmia</i>]	Euclidieae I	4	—	—
<i>Synstemon</i> Botsch.	—	—	—	—	Anchonieae I	2	—	—
<i>Syrenia</i> Andrz. ex Besser	Hesperideae	Hesperideae-Hesperidinae	—	Camelineae, <i>Syrenia</i> [= <i>Erysimum</i>]	Camelineae	—	—	—
<i>Tauscheria</i> Fisch. ex DC.	Euclidieae	Sisymbrieae-Isatidinae	—	Isatideae	—	1	<i>x</i> = 7	<i>n</i> = 7; <i>2n</i> = 12, 14

Table 2. Continued.

Genus	Schulz (1936)	Janchen (1942)	Al-Shehbaz (1988)	Al-Shehbaz et al. (2006)	Present study	No. of species	Base chromosome no. (x)	Chromosome no. (n; 2n)
<i>Tetracme</i> Bunge	Matthioleae	Hesperideae-Matthiolinae	—	Euclidieae	Euclidieae I	10	x = 14	n = 14; 2n = 14, 28
<i>Texiera</i> Jaub. & Spach	Euclidieae	Sisymbrieae-Isatidinae	—	Isatideae, <i>Texiera</i> [= <i>Glastaria</i> Boiss.]	—	—	—	—
<i>Thelypodopsis</i> Rydb.	Hesperideae	Sisymbrieae-Thelypodinae	Thelypodieae	Schizopetaleae	Schizopetaleae	17	x = 10, 11	n = 10, 11
<i>Thelypodium</i> Endl.	Hesperideae	Sisymbrieae-Thelypodinae	Thelypodieae	Schizopetaleae	Schizopetaleae	19	x = 13	n = 13
<i>Veselskya</i> Opiz	Matthioleae, <i>Veselskya</i> [= <i>Pyramidium</i> Boiss.]	Hesperideae-Matthiolinae	Anchonieae	Euclidieae	—	1	—	—
<i>Zerdana</i> Boiss.	Hesperideae	Hesperideae-Hesperidinae	Anchonieae	Anchonieae	Anchonieae I	1	x = 7	2n = 14
<i>Zuvanda</i> (Dvorák) Askerova	—	—	—	—	Unresolved	3	—	—

one evolutionary center for the tribe (as Hesperideae) and central Asia as another. In contrast, Al-Shehbaz (1988) suggested that the occurrence of *Parrya* in the New World more likely represents a recent migration event given that all related genera occur in Central Asia and the greatest diversity of the genus occurs in that part of the world.

The tribe Chorisporaee is exclusively Asian and includes *Chorispora* R. Br. ex DC. and *Diptychocarpus* Trautv. The tribe is characterized by the presence of multicellular-multiseriate glands, the lack of branched trichomes and the presence of connivent stigmas, moniliform fruits breaking into 1-seeded, corky segments, and erect sepals forming a closed calyx (Table 1). Hayek (1911) and Schulz (1936) had also associated *Chorispora* with *Diptychocarpus*, whereas Dvorák (1972) had suggested that *Chorispora* and *Sterigmotemum* were closely related and derived from a common ancestor. Considering the indument and floral morphology, Jacquemoud (1988: 145) suggested that *Chorispora* was instead close to *Hesperis*. In the *ndhF*-based phylogenetic study of Beilstein et al. (2006), *Chorispora* (1 species) and *Diptychocarpus* (1 species) formed a well-supported clade.

As delimited by Al-Shehbaz et al. (2006), the tribe Euclidieae includes about 25 genera and some 150 species. It is primarily Eurasian and north and east African, with only seven species of *Braya* Sternb. & Hoppe found in North America. The tribe is characterized by the lack of multicellular glands and the presence of branched trichomes (rarely simple or absent), and entire or 2-lobed stigmas (Table 1). The *ndhF*-based phylogenetic study of Beilstein et al. (2006) supported a Euclidieae clade with two well-resolved subclades. The first included *Braya*, *Dilophia* Thomson, *Christolea* Cambess., and *Shangrilaia* Al-Shehbaz, J. P. Yue & H. Sun, and the second subclade included *Desideria* Pamp., *Euclidium* R. Br., *Malcolmia* R. Br., *Neotorularia* Hedge & J. Léonard, *Rhammatophyllum* O. E. Schulz, *Sisymbriopsis* Botsch. & Tzvelev, *Solms-laubachia* Muschl., and *Tetracme* Bunge.

The primary objective of this paper was to determine the tribal limits, monophyletic status, and phylogenetic intra-tribal relationships of genera within the related tribes Anchonieae, Chorisporaee, Euclidieae, and Hesperideae as currently defined by Al-Shehbaz et al. (2006) based on a more comprehensive sampling of genera and species than has been published to date. The monophyletic status of genera was also evaluated where multiple congeneric species sampling allowed. Sequences of the internal transcribed spacers of nuclear ribosomal DNA and the 5.8S rRNA gene (collectively, ITS) from a broad analysis of 118 species were used to assess relationships. The ITS region is

currently the most widely used marker in phylogenetic analyses of the Brassicaceae (Koch, 2003; Koch et al., 2003; Warwick et al., 2002, 2004a, 2004b), and a comprehensive, global ITS-based phylogeny for the majority of Brassicaceae species has recently been published (Bailey et al., 2006).

MATERIALS AND METHODS

PLANT MATERIAL

Collection data, taxa, voucher numbers, and Genbank accession numbers are listed in Table 3. Ingroup species (101 species, 131 accessions) were selected based on available material and published sequences to represent the four tribes under study: Anchonieae, Chorisporeae, Euclidieae, and Hesperideae. Following the tribal delimitations outlined in Al-Shehbaz et al. (2006), 10 of the 12 genera assigned to the Anchonieae, 19 of the 25 genera assigned to the Euclidieae, and all genera assigned to the Chorisporeae (2 genera) and Hesperideae (1 genus) were included in the present study. Also included were representatives from seven other tribes, defined in Al-Shehbaz et al. (2006), to which previous members of the Anchonieae have been reassigned. These included 17 species (21 accessions) in the Arabideae, Camelinae, Cardamineae, Cochlearieae, Schizopetaleae, Sisymbrieae, and Thlaspidiae. Although only poor resolution of the backbone phylogeny of the family Brassicaceae has been obtained with analyses of both ITS (Bailey et al., 2006) and *ndhF* data (Beilstein et al., 2006), these studies have supported the recognition of monophyletic clades corresponding to tribal groupings. Each of the latter seven tribes was supported as a monophyletic clade in previous ITS (Bailey et al., 2006) and *ndhF* studies (Beilstein et al., 2006). Multiple accessions were available for 29 of the species studied (Table 3). Generic types are indicated by an asterisk besides species name. The total ingroup included 118 species and 152 accessions; samples from 73 species (95 accessions) were sequenced in this study and the remainder were obtained from Genbank (Table 3). *Aethionema arabicum* (L.) Andr. ex O. E. Schulz in Engler & Prantl was selected as the outgroup on the basis of previous molecular phylogenetic analyses, indicating that Aethionemeae Al-Shehbaz, Beilstein & E. A. Kellogg are the most basal tribe in the family (Al-Shehbaz et al., 2006; Bailey et al., 2006; Beilstein et al., 2006; Koch, 2003; Koch et al., 2007).

DNA EXTRACTION, AMPLIFICATION, AND SEQUENCING

DNA was extracted from approximately 100 mg or less of dried leaf tissue using Bio 101 FastDNA Kit

(Qbiogene, Carlsbad, California, U.S.A.) following manufacturer's instructions. All polymerase chain reaction (PCR) amplifications were performed using Ready-To-Go PCR Beads (Amersham Pharmacia Biotech, Piscataway, New Jersey, U.S.A.) and approximately 50 ng of genomic DNA in a total of 25 µL on a Thermolyne Amplitron thermocycler (Technique, Princeton, New Jersey, U.S.A.). The entire ITS region (including ITS-1 and ITS-2 of nuclear ribosomal DNA and the 5.8S rRNA gene) was amplified and sequenced as a single fragment using the primers ITS-1 18S described by O'Kane et al. (1997) and ITS-4 (White et al., 1990). The PCR amplification, purification, and sequencing followed Warwick et al. (2004a, b). No cloning of the ITS region was done, as there was no evidence of the presence of two or more ITS sequences in the same individual, as seen for example in *Braya* (Warwick et al., 2004a), where nucleotide additivity or the presence of more than one peak was observed at a particular position in a sequence.

DATA SCORING AND ANALYSIS

The sequences were automatically assembled using the software ASSEMBLYLIGN (Kodak, Newhaven, Connecticut, U.S.A.), aligned using the Clustal method in MEGALIGN (DNA Star, Madison, Wisconsin, U.S.A.), and further aligned by eye. Sequences of ITS are deposited in Genbank (Table 3), and the full alignment of the ITS data set is available in TreeBASE (www.treebase.org; study accession number SN2686, Pin No. 15130). Boundaries of the ITS-1 and ITS-2 regions were determined by comparisons with the published sequences of *Arabidopsis thaliana* (L.) Heynh. (O'Kane et al., 1997) and *Sisymbrium irio* L. (Warwick et al., 2002). Insertions and deletions (indels) in the ITS region were treated as missing data to retain phylogenetic information from taxa not having the deletion.

Maximum parsimony analyses of 148 (5 of the 152 sequences in the ingroup were identical to that of other accessions as indicated in Fig. 1, and there was one outgroup accession) aligned ITS sequences were conducted using the computer program PAUP*, version 4.0b10 (Swofford, 2002). The most parsimonious trees were generated using the heuristic search algorithm, with tree bisection-reconnection (TBR) branch-swapping, equal-weighted characters, with gaps treated as missing data, 100 random additions of the sampled taxa, and 100 trees saved per replicate. A second heuristic search was conducted using the 50% majority-rule tree obtained in the above analysis as the starting tree and the replicate allowed to swap to completion using the TBR option, with maximum tree number set at 40,000. Statistics relating to the

Table 3. Taxa sequenced for ITS, with collector and collection number for each voucher specimen and geographical origin of the specimen. The taxonomy followed the most recent family-wide Brassicaceae species checklist (Warwick et al., 2006a).

Taxon*	Collector and No. (Herbarium*); or Citation; or Genbank authors (names in full), unpubl., row 1, Voucher number in row 2	Geographical origin	Genbank Accession No.
1. <i>Anchonium billardieri</i> DC.*	<i>Mouterden 10791</i> (G)	Lebanon	DQ357512
2. <i>Anchonium billardieri</i>	<i>Reese s.n.</i> , 18 May 1933 (G)	Lebanon	DQ357513
3. <i>Anchonium elichrysifolium</i> subsp. <i>canescens</i> (Hausskn. & Bornm. ex Bornm.) Coode & Cullen	<i>Sorger 69–53–7</i> (G)	Turkey	DQ357514
4. <i>Anchonium elichrysifolium</i> subsp. <i>elichrysifolium</i> (DC.) Boiss.	<i>Davis 15976</i> (G)	Turkey	DQ357515
5. <i>Anchonium elichrysifolium</i> subsp. <i>elichrysifolium</i>	<i>Davis & Hedge 31143</i> (G)	Turkey	DQ357516
6. <i>Anchonium elichrysifolium</i> subsp. <i>villosum</i> Coode & Cullen	<i>Davis & Polunin 23460</i> (G)	Turkey	DQ357517
7. <i>Aubrieta deltoidea</i> (L.) DC.*	Koch et al., 1999a	—	AJ232909‡
8. <i>Aubrieta parviflora</i> Boiss.	<i>Al-Shehbaz et al. 7535</i> (MO)	Iraq	DQ357518
9. <i>Blennodia pterosperma</i> (J. M. Black) J. M. Black	<i>Weber 9251</i> (MO)	Australia	DQ357519
10. <i>Braya humilis</i> (C. A. Mey.) B. L. Rob.	Warwick et al., 2004a	Canada (N.W.T.)	AY353111‡
11. <i>Braya humilis</i>	Warwick et al., 2004a	China	AY353123‡
12. <i>Chorispora bungeana</i> Fisch. & C. A. Mey.	<i>Al-Shehbaz 9237</i> (MO)	Kazakhstan	DQ357520
13. <i>Chorispora bungeana</i>	<i>Gibbons 0622</i> (MO)	Afghanistan	DQ357521
14. <i>Chorispora macropoda</i> Trautv.	<i>Gibbons 0407</i> (MO)	Afghanistan	DQ357522
15. <i>Chorispora sibirica</i> (L.) DC.	<i>Skvortsov s.n.</i> , 14 July 1989 (MO)	U.S.S.R. (Altai)	DQ357523
16. <i>Chorispora sibirica</i>	<i>Al-Shehbaz 9469</i> (MO)	Kazakhstan	DQ357524
17. <i>Chorispora tashkorganica</i> Al-Shehbaz, T.Y. Cheo, L. L. Lu & G. Yang	<i>Bartholomew et al. 8369</i> (MO)	China	DQ357525
18. <i>Chorispora tenella</i> (Pall.) DC.*	<i>Henderson 94–190</i> (MO)	U.S.A. (Colorado)	DQ357526
19. <i>Chorispora tenella</i>	<i>Kent 11</i> (MO)	U.S.A. (Colorado)	DQ357527
20. <i>Cithareloma lehmannii</i> Bunge*	<i>Antonio 207</i> (MO)	Iran	DQ357528
21. <i>Clausia aprica</i> (Stephan) Korn.-Trotzky*	<i>Schischkin s.n.</i> , 1 June 1931 (LE)	Kazakhstan	DQ357529
22. <i>Clausia aprica</i>	Bleeker, Hurka, Oyuntsetseg et al., unpubl. OSBU 10099	Mongolia	AY558938.‡ AY558966‡
23. <i>Clausia aprica</i>	Bleeker, Hurka, Oyuntsetseg et al., unpubl. OSBU 10366	Mongolia	AY558937.‡ AY558965‡
24. <i>Clausia kasakhorum</i> Pavlov	<i>Mechankora 380</i> (LE)	Kazakhstan	DQ357530
25. <i>Cryptospora falcata</i> Kar. & Kir.*	<i>Rechinger 55502</i> (G)	Iran	DQ357531
26. <i>Cryptospora falcata</i>	<i>Podlech 21401</i> (G)	Afghanistan	DQ357532
27. <i>Desideria incana</i> (Ovcz.) Al-Shehbaz	Mummenhoff & Muehlhausen, unpubl. Isolate 1031	South Africa	AJ628335.‡ AJ628336‡
28. <i>Desideria prolifera</i> (Maxim.) Al-Shehbaz	Mummenhoff & Muehlhausen, unpubl. Isolate 1033	South Africa	AJ628333.‡ AJ628334‡
29. <i>Diceratella inermis</i> Jonsell	<i>Gilbert & Thulin 1573</i> (MO)	Kenya	DQ357533
30. <i>Dichasianthus subtilissimus</i> (M. Pop.) Ovcz. & Junussov*	Warwick et al., 2004a	Tajikistan	AY353169‡
31. <i>Diptychocarpus strictus</i> (Fisch. ex M. Bieb.) Trautv.*	<i>Podlech & Jarmal 30135</i> (G)	Afghanistan	DQ357534
32. <i>Diptychocarpus strictus</i>	<i>Anders 5502</i> (G)	Afghanistan	DQ357535
33. <i>Dontostemon elegans</i> Maxim.	Bleeker, Hurka, Oyuntsetseg et al., unpubl. OSBU 14740	Mongolia	AY558928.‡ AY558956‡
34. <i>Dontostemon integrifolius</i> (L.) Ledeb.*	<i>Ikonnikor Galitzky 1289</i> (LE)	Mongolia	DQ357536
35. <i>Dontostemon integrifolius</i>	Bleeker, Hurka, Oyuntsetseg et al., unpubl. OSBU 10217	Mongolia	AY558932.‡ AY558960‡
36. <i>Dontostemon integrifolius</i>	Bleeker, Hurka, Oyuntsetseg et al., unpubl. OSBU 12308	Mongolia	AY558926.‡ AY558954‡

Table 3. Continued.

Taxon*	Collector and No. (Herbarium*); or Citation; or Genbank authors (names in full), unpubl., row 1, Voucher number in row 2	Geographical origin	Genbank Accession No.
37. <i>Eremobium aegyptiacum</i> (Spreng.) Asch.* in Boiss	Charpin 24682 (G)	United Arab Emir.	DQ357537
38. <i>Eremobium longisiliquum</i> (Coss.) Boiss.	Podlech 35381 (G)	Algeria	DQ357538
39. <i>Erysimum asperum</i> (Nutt.) DC.	Roy (2001)	—	AF183098‡
40. <i>Erysimum canum</i> (Pill. & Mitterp.) Polatschek [= <i>Syrenia cana</i> (Piller & Mitterp.) Neilr.]	Bano s.n., 21 June 1946 (DAO)	Hungary	DQ357539
41. <i>Erysimum capitatum</i> (Douglas ex Hook.) Greene	Davis 1006 (MO)	U.S.A. (Arizona)	DQ357540
42. <i>Erysimum capitatum</i>	Hong et al., 2003	—	AY254534‡
43. <i>Erysimum cheiri</i> (L.) Crantz	Hong et al., 2003	—	AY254538‡
44. <i>Erysimum majellense</i> Polatschek	Heenan et al., 2002	—	AY028602‡
45. <i>Erysimum repandum</i> L.	Yatskievych & Yatskievych 91 81 (MO)	U.S.A. (Missouri)	DQ357541
46. <i>Erysimum scoparium</i> Wettst.	Heenan et al., 2002	—	AY028604‡
47. <i>Erysimum sisymbrioides</i> C. A. Mey.	Al-Shehbaz et al. 9459 (MO)	Kazakhstan	DQ357542
48. <i>Erysimum witmannii</i> Zaw.	Francisco-Ortega et al., 1999	Romania	AF040000,‡ AF040043‡
49. <i>Euclidium syriacum</i> (L.) R. Br. in W. T. Aiton*	Mariana Cirta s.n., 24 May 1973 (DAO)	Romania	DQ357543
50. <i>Euclidium syriacum</i>	McCaskill 712 (DAO)	U.S.A. (California)	DQ357544
51. <i>Goldbachia laevigata</i> (M. Bieb.) DC.*	Rechinger 51383 (G)	Iran	DQ357545
52. <i>Goldbachia laevigata</i>	Rechinger 39424 (G)	Iran	DQ357546
53. <i>Hesperidanthus argillaceus</i> (S. L. Welsh & N. D. Atwood) Al-Shehbaz [= <i>Schoenocrambe argillacea</i> (S. L. Welsh & N. D. Atwood) Rollins]	Warwick et al., 2002	U.S.A. (Utah)	AF531611‡
54. <i>Hesperis laciniata</i> All. [= <i>Hesperis laciniata</i> subsp. <i>spectabilis</i> (Jord.) Rouy & Foucaud]	Mummenhoff & Muehlhausen, unpubl. Isolate 9700821000	South Africa	AJ628315,‡ AJ628316‡
55. <i>Hesperis matronalis</i> L.*	Merello et al. 2269 (MO)	Georgia	DQ357547
56. <i>Hesperis matronalis</i>	Franzke et al., 2004	—	AY546108,‡ AY546137‡
57. <i>Hesperis matronalis</i>	Mummenhoff & Muehlhausen, unpubl. Isolate 9400193000	South Africa	AJ628313,‡ AJ628314‡
58. <i>Hesperis sibirica</i> L.	Stydzienkena s.n., 30 May 1997 (MO)	Russia (Siberia)	DQ357548
59. <i>Hesperis sibirica</i>	Skvortsov & Blokhina s.n., 31 May 1983 (MO)	Russia	DQ357549
60. <i>Hesperis voronovii</i> N. Busch [= <i>Hesperis</i> <i>matronalis</i> subsp. <i>voronovii</i> (N. Busch) P. W. Ball]	Merello et al. 2190 (MO)	Georgia	DQ357550
61. <i>Iskandera alaica</i> (Korsh.) Botsch. & Vved.	Idarova & Garboshnova s.n., 3 June 1964 (LE)	Tajikistan	DQ357551
62. <i>Iskandera hissarica</i> N. Busch*	Chukavina & Ameerukhamedov 10344 (LE)	Tajikistan	DQ357552
63. <i>Iskandera hissarica</i>	Egorova 2252 (LE)	Tajikistan	DQ357553
64. <i>Leiospora eriocalyx</i> (Regel & Schmalh.) F. Dvorák	Bartholomew et al. 8449 (MO)	China	DQ357554
65. <i>Leiospora exscapa</i> (C. A. Mey.) F. Dvorák*	Bleeker, Hurka, Oyuntsetseg et al., unpubl. ALT B175	Russia (Altai Mtns.)	AY558939,‡ AY558967‡
66. <i>Leiospora pamirica</i> (Botsch. & Vved.) Botsch. & Pachom.	Al-Shehbaz 8368 (MO)	China	DQ357555
67. <i>Leiospora pamirica</i>	Mummenhoff & Muehlhausen, unpubl. Isolate 1037	South Africa	AJ628329,‡ AJ628330‡

Table 3. Continued.

Taxon*	Collector and No. (Herbarium ^a); or Citation; or Genbank authors (names in full), unpubl., row 1, Voucher number in row 2	Geographical origin	Genbank Accession No.
68. <i>Leptaleum filifolium</i> (Willd.) DC.*	<i>Rechinger 54714</i> (G)	Iran	DQ357556
69. <i>Malcolmia africana</i> (L.) R. Br. in W. T. Aiton	<i>Franklin 1354</i> (MO)	U.S.A. (Utah)	DQ357557
70. <i>Malcolmia africana</i>	<i>Harris 1892</i> (UVSC)	U.S.A. (Utah)	AY237307‡
71. <i>Malcolmia karelinii</i> Lipsky	<i>Al-Shehbaz 9405</i> (MO)	Kazakhstan	DQ357558
72. <i>Malcolmia littorea</i> (L.) R. Br. in W. T. Aiton	<i>Leadlay & Petty 376</i> (MO)	Portugal	DQ357559
73. <i>Malcolmia orsiniana</i> (Ten.) Ten.	<i>Evrard 10.828</i> (MO)	Greece	DQ357560
74. <i>Malcolmia triloba</i> (L.) Spreng. [= <i>Malcolmia lacera</i> (L.) DC.]	<i>Lewalle 13005</i> (MO)	Morocco	DQ357561
75. <i>Maresia nana</i> (DC.) Batt.*	<i>Raus & Schiers 16713</i> (MO)	Greece	DQ357562
76. <i>Mathewsia foliosa</i> Hook. & Arn.*	<i>Landrum & Landrum 9837</i> (MO)	Chile	DQ357563
77. <i>Matthiola capiomontana</i> (Durieu) Pomel [= <i>Lonchophora capiomontana</i> Durieu*]	<i>Faurel s.n.</i> , 10 May 1954 (G)	Algeria	DQ357564
78. <i>Matthiola farinosa</i> Bunge ex Boiss.	<i>Makhmedev & Zadorozhnuk</i> 212 (MO)	Turkmenistan	DQ357565
79. <i>Matthiola fruticulosa</i> (L.) Maire	<i>Lewalle 9803</i> (MO)	Morocco	DQ357566
80. <i>Matthiola incana</i> (L.) R. Br.*	Mummenhoff & Muehlhausen, unpubl. Isolate 403	—	AJ628339,‡ AJ628340‡
81. <i>Matthiola lunata</i> DC.	Mummenhoff & Muehlhausen, unpubl. Isolate 355	—	AJ628341,‡ AJ628342‡
82. <i>Matthiola parviflora</i> (Schousb.) R. Br.	<i>Bramwell et al. 210</i> (MO)	Morocco	DQ357567
83. <i>Matthiola parviflora</i>	<i>Miller et al. 923</i> (MO)	Morocco	DQ357568
84. <i>Microstigma brachycarpum</i> Botsch.	<i>Liu & Zhang 93005</i> (MO)	China	DQ357569
85. <i>Morettia canescens</i> Boiss.	<i>Humbles 10073</i> (MO)	Saudi Arabia	DQ357570
86. <i>Morettia parviflora</i> Boiss.	<i>Gasperetti PG435</i> (MO)	Saudi Arabia	DQ357571
87. <i>Morettia philaeana</i> DC.*	<i>Goodman s.n.</i> , 28 Feb. 1985 (MO)	Egypt	DQ357572
88. <i>Neotorularia dentata</i> (Freyn & Sint.) Hedge & J. Léonard	Warwick et al., 2004a	Pakistan	AY353158‡
89. <i>Neotorularia korolkowii</i> (Regel & Schmalh.) Hedge & J. Léonard	Warwick et al., 2004a	Kazakhstan	AY353156‡
90. <i>Neotorularia torulosa</i> (Desf.) Hedge & J. Léonard*	Warwick et al., 2004a	Iran	AY353167‡
91. <i>Notoceras bicornes</i> (Aiton) Amo*	<i>Miller et al. 386</i> (MO)	Morocco	DQ357573
92. <i>Notothlaspi australe</i> Hook. f.*	Mitchell & Heenan, 2000	New Zealand	AF100689‡
93. <i>Notothlaspi rosulatum</i> Hook. f.	Mitchell & Heenan, 2000	New Zealand	AF100690‡
94. <i>Notothlaspi</i> sp. indet.	Mitchell & Heenan, 2000	New Zealand	AF110691‡
95. <i>Oreoloma matthioides</i> (Franch.) Botsch.*	<i>Ma & Liu 83</i> (PE)	China	DQ357574
96. <i>Oreoloma sulfureum</i> Botsch.	<i>Potanin s.n.</i> , 13 May 1877 (LE)	China (Mongolia)	DQ357575
97. <i>Oreoloma violaceum</i> Botsch.	<i>Guan Kejia 574</i> (PE)	China	DQ357576
98. <i>Parolinia intermedia</i> Svent. & Bramwell	<i>Bramwell & Sventinius 1453</i> (MO)	Canary Islands	DQ357577
99. <i>Parrya asperrima</i> (B. Fedtsch.) Popov	<i>Botschantsev 438</i> (LE)	Kazakhstan	DQ357578
100. <i>Parrya pulvinata</i> M. Pop. in Komarov	<i>Botschantsev 202</i> (LE)	Kazakhstan	DQ357579
101. <i>Parrya turkestanica</i> (Korsh.) N. Busch	<i>Yunussov 6479</i> (LE)	Tajikistan	DQ357580
102. <i>Pseudocamelina glaucophylla</i> (DC.) N. Busch*	<i>Schmid 5420</i> (G)	Iran	DQ357581
103. <i>Pseudocamelina glaucophylla</i>	<i>Rechinger 47361</i> (G)	Iran	DQ357582
104. <i>Rhammatophyllum afghanicum</i> (Rech. f.) Al-Shehbaz & O. Appel	<i>Rechinger 36303</i> (B)	Afghanistan	DQ357583
105. <i>Rhammatophyllum frutex</i> Botsch. & Vved.	<i>Karamycheva et al. 4090</i> (LE)	Kazakhstan	DQ357584

Table 3. Continued.

Taxon*	Collector and No. (Herbarium*); or Citation; or Genbank authors (names in full), unpubl., row 1, Voucher number in row 2	Geographical origin	Genbank Accession No.
106. <i>Rhammatophyllum gaudanense</i> (Litv.) Al-Shehbaz & O. Appel	<i>Nikitin et al. s.n.</i> , 18 June 1964 (LE)	Turkmenistan	DQ357585
107. <i>Rhammatophyllum ghoranum</i> (Rech. f.) Al-Shehbaz & O. Appel	<i>Palmer 110</i> (K)	Afghanistan	DQ357586
108. <i>Rhammatophyllum kamelinii</i> (Botsch.) Al-Shehbaz & O. Appel	<i>Kamelin & Darijmaa 331</i> (MO)	Mongolia	DQ357587
109. <i>Rhammatophyllum pachyrhizum</i> (Kar. & Kir.) O. E. Schulz*	<i>Kamaeshiva et al. 4178</i> (LE)	Kazakhstan	DQ357588
110. <i>Sisymbriopsis mollipila</i> (Maxim.) Botsch.	Warwick et al., 2004a	China	AY353157‡
111. <i>Sisymbriopsis yechengnica</i> (C. H. An) Al-Shehbaz et al.	Warwick et al., 2004a	China	AY353161‡
112. <i>Solms-laubachia eurycarpa</i> (Maxim.) Botsch.	<i>Ho et al. 2847</i> (MO)	China	DQ357589
113. <i>Solms-laubachia retropilosa</i> Botsch.	<i>Al-Shehbaz 9363</i> (MO)	China	DQ357590
114. <i>Sterigmostemum acanthocarpum</i> (Fisch. & C. A. Mey.) Kuntze	<i>Rechinger et al. 5318</i> (G)	Iran	DQ357591
115. <i>Sterigmostemum longistylum</i> (Boiss.) Kuntze	<i>Rechinger 46634</i> (G)	Iran	DQ357592
116. <i>Sterigmostemum purpurascens</i> (Boiss.) Kuntze	<i>Léonard 5793</i> (G)	Iran	DQ357593
117. <i>Sterigmostemum purpurascens</i>	<i>Rechinger 28025</i> (G)	Pakistan	DQ357594
118. <i>Sterigmostemum ramosissimum</i> (O. E. Schulz) Rech. f.	<i>Franskeritch s.n.</i> , 14 June 1972 (G)	Turkmenia	DQ357595
119. <i>Sterigmostemum ramosissimum</i>	<i>Rechinger 52318</i> (G)	Iran	DQ357596
120. <i>Sterigmostemum sulphureum</i> (Banks & Sol.) Bornm.	<i>Dep. Bot., U. Bagd. s.n.</i> , 20 Mar. 1958 (G)	Iraq	DQ357597
121. <i>Sterigmostemum sulphureum</i>	<i>Mouterde 8320</i> (G)	Lebanon	DQ357598
122. <i>Strigosella brevipes</i> (Bunge) Botsch.	Bleeker, Hurka, Oyuntsetseg, et al., unpubl. ALTB	Mongolia	AY558940,‡ AY558968‡
123. <i>Synstemon petrovii</i> Botsch.*	<i>Petrov s.n.</i> , 29 June 1958 (LE)	China	DQ357599
124. <i>Tetracme contorta</i> Boiss.	<i>Anders 5560</i> (G)	Afghanistan	DQ357600
125. <i>Tetracme contorta</i>	<i>Podlech 20408</i> (G)	Afghanistan	DQ357601
126. <i>Tetracme quadricornis</i> (Stephan) Bunge*	<i>Koelz 6194</i> (G)	India (Ladakh)	DQ357602
127. <i>Tetracme quadricornis</i>	<i>Rechinger 16836</i> (G)	Afghanistan	DQ357603
128. <i>Tetracme secunda</i> Boiss.	<i>Rechinger 33439</i> (G)	Afghanistan	DQ357604
129. <i>Zerdana anchonioides</i> Boiss.*	<i>Jacquemoud 7105</i> (G)	Iran	DQ357605
130. <i>Zuvanda crenulata</i> (DC.) R. K. Askerova	<i>Bertschinger s.n.</i> , 23 May 1935 (G)	Israel	DQ357606
131. <i>Zuvanda exacoides</i> (DC.) R. K. Askerova	<i>Pabot s.n.</i> , 11 May 1955 (G)	Lebanon	DQ357607
Arabideae			
132. <i>Arabis alpina</i> L.	O’Kane & Al-Shehbaz, 2003	Romania	AF137559‡
133. <i>Arabis blepharophylla</i> Hook. & Arn.	Koch et al., 1999a	—	AJ232903‡
134. <i>Arabis blepharophylla</i>	Koch et al., 1999a	—	AJ232904‡
135. <i>Arabis hirsuta</i> (L.) Scop.	Koch et al., 1999a	—	AJ232885‡
136. <i>Arabis hirsuta</i>	Koch et al., 1999a	—	AJ232886‡
137. <i>Arabis procurrens</i> Waldst. & Kit.	Koch et al., 1999a	—	AJ232916‡
138. <i>Arabis procurrens</i>	Koch et al., 1999a	—	AJ232917‡
139. <i>Pseudoturritis turrita</i> (L.) Al-Shehbaz	Koch et al., 1999a	—	AJ232905‡
140. <i>Pseudoturritis turrita</i>	Koch et al., 1999a	—	AJ232906‡
Cardamineae			
141. <i>Cardamine flexuosa</i> With.	Franzke et al., 1998	—	AF077999,‡ AF0778000‡
142. <i>Rorippa palustris</i> (L.) Besser	Franzke et al., 1998	—	AF078021,‡ AF078022‡

Table 3. Continued.

Taxon*	Collector and No. (Herbarium ^a); or Citation; or Genbank authors (names in full), unpubl., row 1, Voucher number in row 2	Geographical origin	Genbank Accession No.
Camelineae			
143. <i>Arabidopsis thaliana</i> (L.) Heynh.	O’Kane et al., 1997	Afghanistan	ATU43225‡
144. <i>Neslia paniculata</i> (L.) Desv.*	O’Kane & Al-Shehbaz, 2003	—	AF137576‡
Cochleariaceae			
145. <i>Cochlearia megalosperma</i> (Maire) R. Vogt	Koch et al., 1999b	—	AF336208.‡ AF336209‡
146. <i>Ionopsidium acaule</i> Rehb.	Koch et al., 1999b	—	AF336210.‡ AF336211‡
Schizopetaleae			
147. <i>Sisymbrium castellanosii</i> O. E. Schulz	Warwick et al., 2006a	Argentina	AY958592‡
148. <i>Thelypodopsis elegans</i> Rydb.*	Warwick et al., 2002	U.S.A. (Colorado)	AF531638‡
149. <i>Thelypodium flexuosum</i> B. L. Rob.	Warwick et al., 2002	U.S.A. (Nevada)	AF531624‡
150. <i>Weberbaueria peruviana</i> (DC.) Al-Shehbaz	Warwick et al., 2002	Bolivia	AF531593‡
Sisymbrieae			
151. <i>Sisymbrium irio</i> L.	Warwick et al., 2002	Belgium	AF534558‡
Thlaspidaceae			
152. <i>Thlaspi arvense</i> L.	Koch & Munmenhoff, 2001	—	AF336152.‡ AF336153‡
OUTGROUP			
Aethionemeae			
153. <i>Aethionema arabicum</i> (L.) Andr. ex O. E. Schulz	Hong et al., 2003	—	AY254539‡

* Type species.
^a Herbarium acronyms (Holmgren et al., 1990).
[‡] Previously published sequences, citation given in second column.

amount of homoplasy in the trees, described in PAUP*, were obtained for each tree, and a strict consensus tree was computed. A total of 1000 bootstrap (Felsenstein, 1985) replicates, respectively, were generated in PAUP* using a full heuristic search, with options ACCTRAN, MULTREES=MULTI, and TBR branch-swapping, and each replicate generated with simple addition sequence of taxa, to test the stability of particular nodes in the parsimony analysis. Pairwise distance ITS sequence divergence was calculated for each accession pair in PAUP* using the Kimura two-parameter model (Kimura, 1980) and the pairwise deletion option for gaps and ambiguous data.

Fifty-six sequence evolution models for maximum-likelihood analyses were tested with the aid of Modeltest v3.08 (Posada & Crandall, 1998) to establish which model of DNA substitution best fits the data. This program compares these maximum-likelihood models in a hierarchical testing framework by calculating the likelihood ratio statistic between different models. Likelihood ratio tests and their associated *P* value compare alternative models of sequence evolution and improvement in fit with increasing model complexity. A heuristic maximum-likelihood search was then con-

ducted in PAUP* using parameters determined for the best model of sequence evolution, “as is” sequence addition, and TBR swapping.

RESULTS

A total of 119 species (153 accessions), including the outgroup, were used in the analysis (Table 3). The resulting multiple alignment of the ITS region, including the 5.8S gene, was 869 bp long. Of these, 398 bp were parsimony uninformative, 108 bp were variable but not parsimony informative, and 363 bp were potentially parsimony informative. The ITS-1 region was variable at 261 of 444 sites (58.8%), the ITS-2 at 190 of 260 sites (73.1%), and the 5.8S gene at only 20 of the 165 sites (12.1%). The alignment required the introduction of several indels, most of which were 1 to 3 bp, with the exception of a 108 bp insertion for *Sterigmostemum acanthocarpum* (Fisch. & C. A. Mey.) Kuntze and *S. sulphureum* (Banks & Soland.) Bornm.; a 27 bp deletion in ITS-1 for *Aubrieta* Adans. species; a 7 bp insert in ITS-2 for *Malcolmia triloba* (L.) Spreng. and *M. littorea* (L.) R. Br.; a 6 bp deletion in ITS-1 for *Anchonium* DC., *Oreoloma*, *Zerdana* Boiss., *Synstemon* Botsch., and

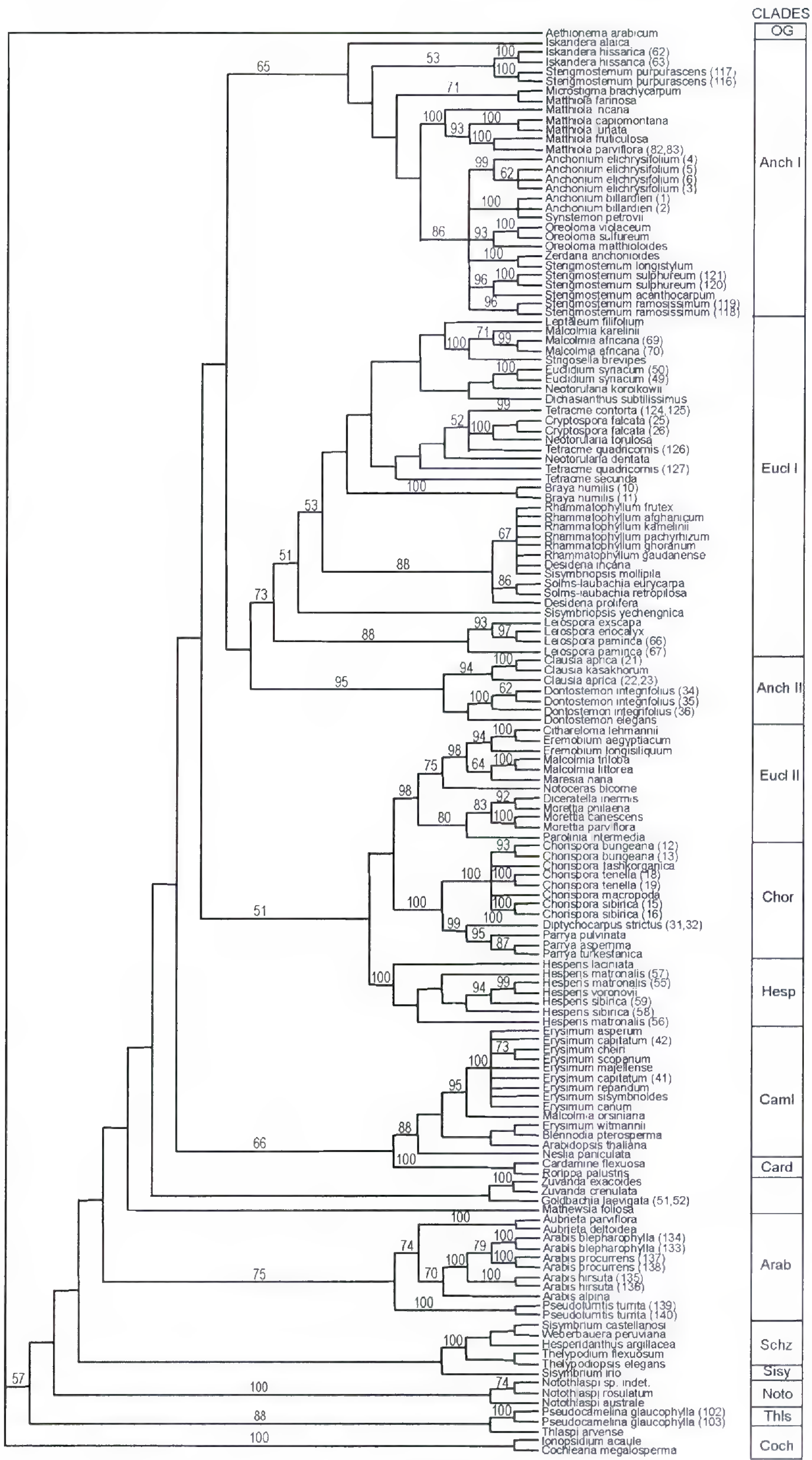


Figure 1. Strict consensus tree of the 34,500 most parsimonious trees generated from analysis in PAUP* of 148 ITS sequences from 121 taxa. Tree length = 2846 (based on 363 parsimony informative characters); consistency index (CI) = 0.273; retention index (RI) = 0.741. Bootstrap values with over 50% support appear above branches. The numbers in parentheses reflect taxon/species ordering in Table 3; all conspecific samples listed within parentheses had identical sequences. Major clades are indicated to the right and include from top to bottom: Outgroup (OG), Anchioideae I (Anch I), Euclidiaceae I (Eucl I), Anchioideae II (Anch II), Euclidiaceae II (Eucl II), Chorisporaceae (Chor), Hesperideae (Hesp), Camelineae (Caml), Cardamineae (Card), Arabideae (Arab), Schizopetaleae (Schz), Sisymbrieae (Sisy), *Notothlaspi* (Noto), Thlaspidaceae (Thls), and Cochleariaceae (Coch).

Sterigmostemum species (excluding *S. purpurascens* (Boiss.) Kuntze); a 6 bp insert in ITS-1 for *Chorispora* species; a 33 bp deletion in ITS-1 for *Diptychocarpus* accessions; a 11 bp deletion for *Parrya* species; a 14 bp and 12 bp insertion in ITS-2 for *Chorispora*, *Diptychocarpus*, and *Parrya*; and a 7 bp insertion in ITS-2 for *Chorispora* and *Parrya*. The length of the ITS region in the ingroup taxa ranged from 597 bp for *Aubrieta* species to 727 bp for *Sterigmostemum acanthocarpum*. The G + C content of taxa in the ingroup averaged 55%.

The equal weight maximum parsimony analysis, based on 148 ITS sequences (each representing a unique sequence) and 363 informative characters, yielded 550 most parsimonious trees of 2846 steps. When the 50% majority-rule tree of the above 550 trees was used as the starting tree in a subsequent heuristic search and then swapped to completion, a total of 34,500 most parsimonious trees were obtained. The strict consensus trees of both analyses were the same. Figure 1 shows the strict consensus tree, with bootstrap values. Several well-supported clades were observed and are listed below in the order they appear in the figure: Anchonieae I (Anch I), Euclidieae I (Eucl I), Anchonieae II (Anch II), Euclidieae II (Eucl II), Chorisporae (Chor), Hesperideae (Hesp), Camelineae (Caml), Cardamineae (Card), Arabideae (Arab), Schizopetaleae (Schz), Sisymbrieae (Sisy), *Notothlaspi* Hook. f. (Noto), Thlaspidiae (Thls), and Cochleariae (Coch) (Fig. 1). Figure 2 shows one of the most parsimonious trees, with the number of characters supporting each clade indicated.

Hierarchical likelihood ratio tests indicated that the best model of DNA substitution for the ITS data is TrN + I + G, which allows for unequal base frequencies, two substitution rates for transitions, one substitution rate for transversions, among site rate heterogeneity allowing some sites to be invariant (I = 0.272), while the rest have rates drawn from a discrete approximation to a gamma distribution, with a single shape parameter, alpha (G = 0.9274). With the TrN + I + G model, base frequencies for A = 0.2597, C = 0.2399, G = 0.2078, T = 0.2926, and substitution rates A-C, A-T, C-G, and G-T = 1.0000, A-G = 2.3031, and C-T = 3.2256. The heuristic search produced a single tree (-ln L = 15673.5) shown in Figure 3 that is nearly identical in topology to the consensus tree of the parsimony analysis. The same major clades were evident, with only minor differences in the positions of *Goldbachia* DC., *Zuvanda* (Dvorák) Askerova, and *Mathewsia* Hook. & Arn. relative to each other. Their tribal positions were also not resolved in the PAUP* analysis.

ITS sequence divergence estimates among species (e.g., corrected pairwise distance values based on the

Kimura two-parameter model) are given in Table 4. Within-clade estimates ranged from 0.33% to 23.5% (mean 11.3%) for the Anchonieae I clade, from 1.6% to 13.3% (mean 8.3%) for the Anchonieae II clade, from 0.66% to 18.7% (mean 7.3%) for the Euclidieae I clade, from 0.16% to 18.4% (mean 11.9%) for the Euclidieae II clade, from 1.99% to 12.9% (mean 7.8%) for the Chorisporae clade, and from 2.8% to 8.7% (mean 5.8%) for the Hesperideae clade. The mean sequence divergence estimates among these six clades were similar, ranging from 13.1% to 20.4%.

In general, multiple accessions of a given species formed single clades. Eleven of 24 genera, where more than two congeneric species were analyzed, were monophyletic (Fig. 1); these included *Aubrieta*, *Chorispora*, *Clausia* Korn.-Trotzky, *Dontostemon*, *Hesperis*, *Leiospora* (C. A. Mey.) F. Dvorák, *Notothlaspi*, *Oreoloma*, *Parrya*, *Solms-laubachia*, and *Zuvanda*.

DISCUSSION

TRIBES/CLADES

The major clades recognized in the ITS analyses correspond very closely to *ndhF*-based clades described in Beilstein et al. (2006), which were used as a basis for a new tribal system for the family. Our results are discussed relative to the corresponding tribal system outlined in Al-Shehbaz et al. (2006).

ANCHONIEAE I

This ITS clade (Figs. 1–3) included *Anchonium*, *Iskandera* N. Busch, *Matthiola*, *Microstigma* Trautv., *Oreoloma*, *Sterigmostemum*, *Synstemon*, and *Zerdana*. The clade was divided into three main subclades: (i) *Anchonium*, *Oreoloma*, *Sterigmostemum* (excluding *S. purpurascens*), *Synstemon*, and *Zerdana* (86% bootstrap support); (ii) *Matthiola*, excluding *M. farinosa* Bunge ex Boiss. (100% bootstrap support); and (iii) *Microstigma* and *Matthiola farinosa* (71% bootstrap support) (Fig. 1). *Matthiola* formed a monophyletic group, with the exclusion of *M. farinosa*. The placement of *Lonchophora* Durieu (represented in this study by *M. capiomontana* (Durieu) Pomel) in synonymy of *Matthiola* (Gowler, 1998; Appel & Al-Shehbaz, 2003) was supported by the ITS data. *Sterigmostemum*, as defined by Jacquemoud (1988), is polyphyletic, with *S. purpurascens* forming a sister taxon to *Iskandera*. The latter species, an annual endemic to Iran and Pakistan, was considered to be segregated from the rest of *Sterigmostemum* (Jacquemoud, 1988) based on the presence of peculiar subglobose glands, white or pale pink flowers, and (as in *Iskandera*) free inner stamens. Nevertheless,

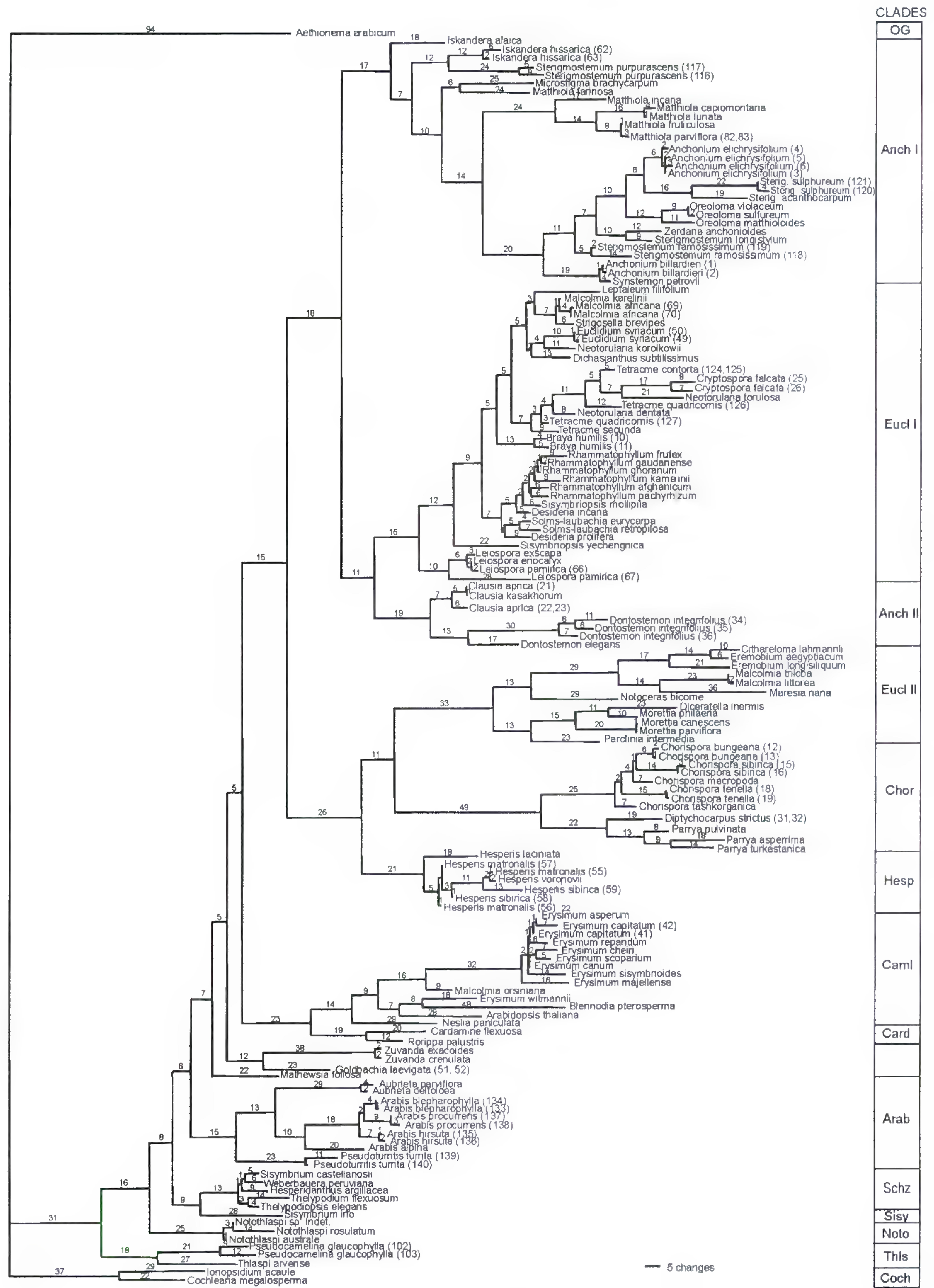


Figure 2. One of the most parsimonious trees, tree length = 2846 (based on 363 parsimony informative characters); CI = 0.273; RI = 0.741. The number of characters supporting each clade is indicated. The numbers in parentheses reflect taxon/species ordering in Table 3; all conspecific samples listed within parentheses had identical sequences. Major clades are indicated to the right and include from top to bottom: Outgroup (OG), Anchonieae I (Anch I), Euclidieae I (Eucl I), Anchonieae II (Anch II), Euclidieae II (Eucl II), Chorisporae (Chor), Hesperideae (Hesp), Camelineae (Caml), Cardamineae (Card), Arabideae (Arab), Schizopetaleae (Schz), Sisymbrieae (Sisy), *Notothlaspi* (Noto), Thlaspidiae (Thls), and Cochlearieae (Coch). *Sterig.* = *Sterigmostemum*.



Figure 3. Maximum likelihood tree generated under the TrN + I + G model of DNA substitution in PAUP* in an analysis of 148 ITS sequences from 121 taxa. The numbers in parentheses reflect taxon/species ordering in Table 3; all conspecific samples listed within parentheses had identical sequences. Major clades are indicated to the right and include: Outgroup (OG), Anchonieae I (Anch I), Euclidieae I (Eucl I), Anchonieae II (Anch II), Camelineae (Cam), Cardamineae (Card), Arabideae (Arab), Euclidieae II (Eucl II), Chorisporaceae (Chor), Hesperideae (Hesp), *Notothlaspi* (Noto), Cochleariaceae (Coch), Schizopetaleae (Schz), Sisymbrieae (Sisy), and Thlaspidaceae (Thls). *Sterig.* = *Sterigmostemum*. The topology of the tree is almost identical to the strict consensus tree (Fig. 1) of the most parsimonious trees with the exception of the relative positions of *Goldbachia*, *Zuvanda*, and *Mathewsia*, whose tribal alignment was also not resolved in the PAUP* analysis.

Table 4. Mean (range) sequence divergence estimates (i.e., corrected pairwise distance values based on Kimura two-parameter model) between and within six clades observed in the present study: Anchonieae I and II, Euclidieae I and II, Chorisporeae, and the Hesperideae.

	Anchonieae I	Anchonieae II	Euclidieae I	Euclidieae II	Chorisporeae	Hesperideae
Anchonieae I	0.1129 (0.0033– 0.2345)					
Anchonieae II	0.1568 (0.1061– 0.2581)	0.0829 (0.0161– 0.1326)				
Euclidieae I	0.1308 (0.0170– 0.2724)	0.1398 (0.0990– 0.2186)	0.0725 (0.0066– 0.1873)			
Euclidieae II	0.1966 (0.1529– 0.3357)	0.2026 (0.1712– 0.3153)	0.1960 (0.1464– 0.3047)	0.1190 (0.0016– 0.1843)		
Chorisporeae	0.1906 (0.1543– 0.3045)	0.1971 (0.1664– 0.2567)	0.2016 (0.1536– 0.3093)	0.1986 (0.1710– 0.2488)	0.0779 (0.0199– 0.1289)	
Hesperideae	0.1947 (0.1267– 0.2702)	0.1879 (0.1505– 0.2340)	0.1942 (0.1424– 0.2493)	0.2040 (0.1423– 0.3154)	0.1960 (0.1465– 0.2762)	0.0580 (0.0282– 0.0869)

trichome morphology, chorology, ecology, habit, and moreover, the lack of affinities with any genus but *Sterigmostemum* within the Hesperidae-Matthiolae group led this author to keep the species in *Sterigmostemum*. In contrast, Léonard (1980) used the peculiarity of *S. purpurascens* as the basis for its placement in the monotypic *Petiniotia* J. Léonard. The ITS data clearly support a closer affiliation of the species with *Iskandera* than with *Sterigmostemum*. The remainder of the *Sterigmostemum* taxa were included in a subclade, with *Anchonium*, *Oreoloma*, *Synstemon*, and *Zerdana*. *Oreoloma*, a genus of three species from China and Mongolia (Botschantzev, 1980) all of which were examined here, formed a monophyletic group (Fig. 1), although the genus has recently been united with *Sterigmostemum* (Kamelin & German, 2001). *Synstemon*, another Chinese endemic (2 species; Al-Shehbaz et al., 2000b), formed a sister group to *Anchonium billardieri* DC. *Zerdana*, a west Iranian endemic (1 species; Jacquemoud, 1985), formed a sister group to *Sterigmostemum longistylum* (Boiss.) Kuntze. The Middle Eastern *Anchonium* (2 species; Jacquemoud, 1984) did not form a monophyletic group.

Although the Anchonieae I has only 65% bootstrap support (Fig. 1), it corresponds well to the Anchonieae sensu Al-Shehbaz et al. (2006), except that it excludes *Dontostemon*, *Parrya*, and *Clausia*. The additional adjustments needed in this tribe are perhaps to transfer *Matthiola farinosa* to *Microstigma* and to recognize the genus *Petiniotia* as distinct from *Sterigmostemum*. Furthermore, contrary to their union

in one genus (Kamelin & German, 2001), *Oreoloma* should continue to be maintained as distinct from *Sterigmostemum*, in particular on the basis of its floral morphology (petals longly clawed, lateral sepals saccate at the base). Indumentum morphology and inner stamens status also show close affinities within the Anchonieae: only members of *Anchonium*, *Oreoloma*, *Sterigmostemum* s. str., and *Zerdana* share the simultaneous occurrence of branched hairs, stalked glands, inner connate stamens, and nothorhizous embryo, whereas *Iskandera* only shares with them branched hairs, stalked glands, and nothorhizous embryo (Jacquemoud, 1988).

Chromosome reports indicated a wide range of numbers for members in this clade, with $x = 5, 6, 7, 8$, and 13 (Table 2; Warwick & Al-Shehbaz, 2006).

ANCHONIEAE II

Clausia (6 species; central Asia, southeastern Europe, and Middle East) and *Dontostemon* (11 species, eastern and central Asia; Al-Shehbaz & Ohba, 2000), which were assigned to the Anchonieae in Al-Shehbaz et al. (2006), formed a well-supported ITS clade (95% support in Fig. 1) distinctly separate from the other Anchonieae. Both genera were monophyletic (Fig. 1) and have a base chromosome number of $x = 7$ (Table 2; Warwick & Al-Shehbaz, 2006). The group would appear to represent a distinct tribe. The central Asian *Pseudoclausia* Popov should be studied in connection with these two genera because of their marked morphological similarities (Appel & Al-Shehbaz, 2003).

EUCLIDIEAE I

This clade (see Figs. 1–3) included *Braya* (13 species; Warwick et al., 2004a), *Cryptospora* Kar. & Kir. (3 species; Botschantzev, 1963b), *Desideria* (12 species; Al-Shehbaz, 2000), *Dichasianthus* Ovcz. & Junussov (1 species), *Euclidium* (2 species), *Leiospora* (6 species), *Leptaleum* DC. (1 species), *Neotorularia* (11 species; Warwick et al., 2004a), *Rhammatophyllum* (10 species; Al-Shehbaz & Appel, 2002), *Sisymbriopsis* (3 species; Al-Shehbaz et al., 1999), *Solms-laubachia* (9 species; Lan & Cheo, 1981; Al-Shehbaz & Yang, 2001; Yue et al., 2006), *Strigosella* Boiss. (as *Malcolmia africana* (L.) R. Br. and *M. karelinii* Lipsky), and *Tetracme* (10 species). All of these were assigned to the Euclidieae by Al-Shehbaz et al. (2006). Previous studies (Warwick et al., 2004a) supported the close relationships among *Braya*, *Dichasianthus*, *Neotorularia* s.l., and *Sisymbriopsis*, and these genera fell in the same tribe defined by Al-Shehbaz et al. (2006) as the Euclidieae. Base chromosome numbers for genera in this clade are $x = 7$ or 14, with the exception of *Rhammatophyllum* with $x = 8$ (Table 2; Warwick & Al-Shehbaz, 2006).

Desideria, *Rhammatophyllum*, *Solms-laubachia*, and *Sisymbriopsis mollipila* (Maxim.) Botsch. formed a well-supported subclade (88% bootstrap support) within the Euclidieae I clade (Figs. 1–3). A similarly well-supported clade (95% bootstrap support) was observed for these four genera in the *ndhF*-based studies of Beilstein et al. (2006). In recent *matK* and *Chs*-based studies (Yue et al., 2006), *Desideria* and *Solms-laubachia* also formed a well-supported clade (100% bootstrap support), along with *Phaeonychium jafrii* Al-Shehbaz; neither *Desideria* nor *Solms-laubachia* were monophyletic, leading these authors to suggest that *Solms-laubachia* be expanded to include *Desideria* and *P. jafrii*. These and the ITS data presented herein suggest that further studies on the generic limits of these related genera are required.

Although the genera *Parolinia* Webb, *Maresia* Pomel, *Morettia* DC., *Cithareloma* Bunge, *Eremobium* Boiss., and *Notoceras* R. Br. were suggested by Al-Shehbaz et al. (2006) to be possible representatives of the Euclidieae, our results clearly support their removal with the Mediterranean *Malcolmia* (including the generic type) into a separate tribal clade (see below). Our studies also support the splitting of both *Sisymbriopsis* sensu Al-Shehbaz et al. (1999) and *Neotorularia* (Léonard, 1986) into at least two genera (for further details, see Warwick et al., 2004a). *Strigosella brevipes* (Bunge) Botsch. formed a well-supported clade with *Malcolmia africana* and *M. karelinii* that was separate from the clade containing European/Mediterranean taxa of *Malcolmia*, which are the most likely sister species to

the type of *Malcolmia* (*M. maritima* (L.) R. Br., material not included). The latter taxa fell outside the Euclidieae as defined herein, whereas those placed in *Strigosella* sensu Botschantzev (1972b) were retained in that tribe. Therefore, our data strongly support the independent recognition of *Strigosella*, a genus reduced to synonymy of *Malcolmia* by nearly all except a few authors (e.g., Townsend, 1980; Czerepanov, 1995). Our results clearly show that the two genera are not closely related and should be assigned to different tribes. *Strigosella* is primarily central and southwest Asian and has non-saccate lateral sepals, stalked and dendritic or forked trichomes, and a base chromosome number of $x = 7$ (Warwick & Al-Shehbaz, 2006). By contrast, *Malcolmia* is Mediterranean and has strongly saccate lateral sepals, sessile stellate or malpighiaceae trichomes, and base chromosome numbers of $x = 8$ or 10 (Warwick & Al-Shehbaz, 2006).

EUCLIDIEAE II

As shown in Figures 1 and 2 and discussed above, the Euclidieae II clade was well defined with 98% bootstrap support, and unrelated to other Euclidieae. It separated into two subclades: the first with 75% bootstrap support included *Cithareloma* (Jafri, 1973), *Eremobium*, *Malcolmia*, *Maresia*, and *Notoceras*, whereas the second subclade had 80% bootstrap support and included the African genera *Diceratella* Boiss. (11 species; Jonsell, 1979), *Morettia* (3 species; Jonsell, 1979; Stork & Wüest, 1980), and *Parolinia* (5 species; Bramwell, 1970). All genera of Euclidieae II were tentatively assigned to the Euclidieae in Al-Shehbaz et al. (2006), but they clearly form a distinct clade separate from the other Euclidieae. Prantl (1891) placed all of the above genera in the tribe Hesperideae subtribe Malcolmiinae, but it is clear that the group should be kept as a distinct tribe, with subtribe Malcolmiinae raised to the tribal rank. Further studies are needed to establish if the two subclades of Euclidieae II merit a subtribal rank.

All three genera in the African subclade have a base chromosome number of $x = 11$; whereas chromosome numbers for the second Eurasian subclade are more variable with $x = 7, 8, 10, 11, 13, 14$ (Table 2; Warwick & Al-Shehbaz, 2006).

CHORISPOREAE

This clade included *Chorispora*, *Diptychocarpus*, and *Parrya* (Figs. 1–3). Both *Chorispora* and *Parrya* formed monophyletic groups. The ITS data are consistent with the separate recognition of this tribe by Al-Shehbaz et al. (2006), which included *Chorispora* (11 species; Al-Shehbaz et al., 2000a) and

Diptychocarpus (1 species). In the *ndhF*-based phylogenetic study of Beilstein et al. (2006), *Chorispora* (1 species) and *Diptychocarpus* (1 species) formed a well-supported clade. *Parrya*, a primarily central Asian genus of ca. 34 species (Botschantzev, 1972a), was not included in the *ndhF* study. Its placement in the Anchonieae with other genera having multicellular-multiseriate glands was recommended by Al-Shehbaz et al. (2006), but ITS analyses clearly support its reassignment to the Chorisporeae. As concluded herein, the genus is firmly assigned to the tribe Chorisporeae instead of the Anchonieae or other tribes. All three genera in this tribe have a base chromosome number of $x = 7$ (Table 2; Warwick & Al-Shehbaz, 2006).

HESPERIDEAE

Hesperis formed a well-supported clade (Figs. 1–3), consistent with its assignment to a separate unigeneric tribe, the Hesperideae, by Al-Shehbaz et al. (2006). As delimited by Prantl (1891), Hayek (1911), and Schulz (1936), the Hesperideae had included many other genera, none of which have uniseriate glands, and de Candolle (1821) had even placed *Hesperis* in the Sisymbrieae. The genus *Hesperis* (46 species) is centered in the Middle East and Europe, with poor representations in northern Africa and central Asia. Chromosome number reports are highly variable for the genus (Table 2; Warwick & Al-Shehbaz, 2006).

TAXA EXCLUDED FROM THE ANCHONIEAE, CHORISPOREAE, EUCLIDIEAE, AND HESPERIDEAE

The studies herein support the exclusion of *Aubrieta*, *Blennodia* R. Br., *Erysimum*, *Goldbachia*, *Hesperidanthus* (B. L. Rob.) Rydb., *Notothlaspi*, *Pseudocamelina* (Boiss.) N. Busch, and *Zuvanda* from the Anchonieae, Chorisporeae, Euclidieae, and Hesperideae. *Aubrieta*, originally assigned to the Matthioleae by Schulz (1936), formed herein a sister clade to *Arabis* L. (Figs. 1–3), consistent with its reassignment to the tribe Arabideae by Janchen (1942) and Al-Shehbaz et al. (2006). Both *Aubrieta* and *Arabis* have base chromosome numbers of $x = 8$ (Warwick & Al-Shehbaz, 2006). Placement of *Aubrieta* in the Arabideae is also consistent with previous ITS-based studies of *A. deltoidea* (L.) DC. (Bailey et al., 2006), *ndhF*-based phylogenetic studies of *A. deltoidea* and *A. parviflora* Boiss. (Beilstein et al., 2006), and the alignment of *A. deltoidea* in a supernetwork multi-gene-based analysis of the family (Koch et al., 2007).

The Australian genus *Blennodia* was originally placed in the Hesperideae by Schulz (1936) and maintained there by Shaw (1965). Al-Shehbaz (1988)

indicated that it should be excluded from this tribe, but its placement is problematic and, thus, was not assigned in Al-Shehbaz et al. (2006). It aligned herein with tribe Camelinae (Fig. 1), forming a loosely supported (less than 50%) clade with *Arabidopsis thaliana* and *Erysimum witmannii* Zaw. in the present analyses.

Erysimum was assigned to the Hesperideae by Schulz (1936) and Janchen (1942), to the Anchonieae by Al-Shehbaz (1988), and later to the Camelinae, along with *Arabidopsis* (DC.) Heynh., by Al-Shehbaz et al. (2006). Placement of *Erysimum* in the Camelinae as observed in the present study (Figs. 1–3) is also consistent with previous ITS (Bailey et al., 2006) and *ndhF* data (Beilstein et al., 2006) for *E. capitatum* (Douglas ex Hook.) Greene. *Erysimum* (with the exception of *E. witmannii*) formed a monophyletic group (100% bootstrap support; Fig. 1), with the inclusion of *Malcolmia orsiniana* (Ten.) Ten. The latter specimen would appear to be correctly identified (as confirmed by Al-Shehbaz), and its inclusion with *Erysimum* requires further analysis of additional accessions. The inclusion in *Erysimum* of *Cheiranthus* L. and *Syrenia* Andr. ex Besser, represented in this study by *E. cheiri* (L.) Crantz and *E. canum* (Piller & Mitterp.) Polatschek, respectively, was supported by the ITS data herein. *Neslia* Desv. (1 species), assigned to the Camelinae in Al-Shehbaz et al. (2006), was closely related to *Erysimum* and *Arabidopsis* and is retained herein in that tribe.

Goldbachia, a Eurasian genus of six species (Botschantzev, 1963a), and *Zuvanda*, a southwest Asian genus of three species (Askerova, 1985), were sister clades in the strict consensus tree shown in Figure 1; the latter clade, however, had less than 50% bootstrap support. Both were placed in the Hesperideae by Schulz (1936) and later in the subtribe Buniadinae DC. of the Sisymbrieae by Janchen (1942), but they were not assigned in Al-Shehbaz et al. (2006). Taxa currently assigned to *Zuvanda* were included in *Malcolmia* (Schulz, 1936), later placed in *Maresia* subgenus *Zuvanda* (Dvorák, 1972), then treated as a separate genus by Askerova (1985), and lastly united with *Moricandia* DC. of the totally unrelated tribe Brassiceae by Dorofeyev (2002). The exclusion of *Zuvanda* from both *Malcolmia* and *Maresia* was supported by our ITS data (Figs. 1–3). The tribal positions of *Goldbachia* and *Zuvanda*, however, are unresolved in the present study; the tribal position of *Goldbachia laevigata* (M. Bieb.) DC. also was not resolved in recent *ndhF*-based phylogenetic studies (Beilstein et al., 2006). Both *Zuvanda* and *Goldbachia* are glabrous and somewhat glaucous annuals with auriculate cauline leaves. They need to be closely studied with members of the tribe Isatideae DC. to determine whether they merit a tribal rank of their own.

Hesperidanthus (5 species), originally assigned to Matthioleae in Schulz (1936) and to Hesperideae subtribe Matthiolinae in Janchen (1942), formed a clade with the New World *Sisymbrium*, *Thelypodium* Endl., *Thelypodopsis* Rydb., and *Weberbaueria* Gilg & Muschl., all members of the tribe Schizopetaleae (Al-Shehbaz et al., 2006; = Thelypodieae Alliance in Warwick et al., 2002, 2006b). The ITS data, herein for *H. argillaceus* (S. L. Welsch & N. D. Atwood) Al-Shehbaz, thus support its assignment to that tribe by Al-Shehbaz et al. (2006). Placement of *Hesperidanthus* in the Schizopetaleae is also consistent with previous ITS data for *H. argillaceus* and *H. linearifolius* (A. Gray) Rydb. (Bailey et al., 2006) and *ndhF* data (Beilstein et al., 2006) for *H. jaegeri* (Rollins) Al-Shehbaz [= *Caulostramina jaegeri* (Rollins) Rollins] and *H. suffrutescens* (Rollins) Al-Shehbaz [= *Glaucocarpum suffrutescens* (Rollins) Rollins]. In contrast, *Mathewsia foliosa* Hook & Arn. did not align with the Schizopetaleae (Al-Shehbaz et al., 2006) or any other tribal clade in the present analyses (Figs. 1–3). The genus *Mathewsia* (10 species) is endemic to South America and uniquely occupies desert habitats. Its generic affinities have been difficult to define (Rollins, 1966) and remain so. A better sampling of the genus, along with several South American genera of the Brassicaceae, is needed to determine its tribal assignment.

Notothlaspi (2 species), a genus endemic to New Zealand, was tentatively assigned to the Euclidieae (Al-Shehbaz et al., 2006) but did not align with any of the tribal clades in this analysis (Figs. 1–3). It had been previously assigned to the Lepidieae (Schulz, 1936; Janchen, 1942). Previous ITS-based molecular studies (Mitchell & Heenan, 2000; Heenan et al., 2002; Bailey et al., 2006) also showed that *Notothlaspi* formed a distinct monophyletic group that was not clearly aligned with any of the tribes studied, including the Lepidieae. The genus has an unusually high chromosome number of $x = 19$ (Table 2; Warwick & Al-Shehbaz, 2006), which would suggest polyploid origins. This genus needs to be studied closely in connection with genera that have similar morphologies, such as *Grammosperma* O. E. Schulz and *Carinavalva* Ising; all three have angustiseptate, many-seeded fruits, and a Southern Hemisphere distribution.

Pseudocamelina, an Iranian genus of three species (Miller, 1978; Appel & Al-Shehbaz, 2003), was originally assigned to the Matthioleae by Schulz (1936) and to the Lepidieae by Janchen (1942). It formed a well-supported clade in Figure 1 with *Thlaspi* L., consistent with its assignment to the Thlaspidieae in Al-Shehbaz et al. (2006) and its tribal placement based on *ndhF*-based studies of *Pseudocamelina campylopoda* Bornm. & Gauba ex Bornm. (Beilstein et al., 2006).

GENERIC MONOPHYLY

Eleven of the 24 genera where more than two congeneric species were analyzed were monophyletic; these included *Aubrieta*, *Chorisporea*, *Clausia*, *Dontostemon*, *Hesperis*, *Leiospora*, *Notothlaspi*, *Oreoloma*, *Parrya*, *Solms-laubachia*, and *Zuvanda*. Twelve genera, *Anchonium*, *Desideria*, *Eremobium*, *Erysimum*, *Iskandera*, *Matthiola*, *Morettia*, *Neotorularia*, *Rhammatophyllum*, *Sisymbriopsis*, *Sterigmostemum*, and *Tetracme*, each fell within a single tribal clade but were not monophyletic. The genus *Malcolmia*, ca. 35 species, was, however, paraphyletic across tribal clades. The species were separated into three clades:

(i) “*Strigosella* subclade”

The two central Asian species, *Malcolmia africana* and *M. karelinii*, formed a distinct clade (100% bootstrap support) with *Strigosella brevipes* and other eastern and central Asian species in the Euclidieae I clade (Fig. 1), including *Neotorularia korolkowii* (Regel & Schmalh.) Hedge & J. Léonard and *Leptaleum filifolium* (Willd.) DC. *Strigosella* (Boissier, 1854) was included in the synonymy of *Malcolmia* in Appel and Al-Shehbaz (2003). The ITS data herein are consistent with its separate recognition from *Malcolmia*.

(ii) “*Erysimum* subclade”

Malcolmia orsiniana, a European species restricted to the mountains of the Balkan peninsula, was included in *Erysimum* in the tribe Camelinaeae clade (Fig. 1). Its inclusion with *Erysimum* requires further analysis of additional accessions.

(iii) “*Malcolmia* subclade”

The two southwestern European taxa, *Malcolmia triloba* (L.) Spreng. and *M. littorea* (L.) R. Br., were sister taxa to *Maresia nana* (DC.) Batt. (= *Malcolmia nana* (DC.) Boiss., and treated as such by Greuter et al., 1986). The three taxa formed a clade with *Cithareloma*, *Eremobium*, and *Notoceras* in a distinctly separate clade (75% bootstrap support) within the Euclidieae II clade (Fig. 1).

SEQUENCE DIVERGENCE

The mean sequence divergence estimates among the six clades, Anchonieae I and II, Euclidieae I and II, Chorisporeae, and Hesperideae, were similar, ranging from 13.1% to 20.4% (Table 4). Based on recent calculations for several Brassicaceae, corrected ITS distance values (Kimura two-parameter model) of 1% correspond to approximately 1 million years (Mya) (Koch & Al-Shehbaz, 2004). Estimated sequence divergence times for the above six clades were similar to those estimated among other tribal groups in the Brassicaceae (14 to 28 Mya; Lysak et al., 2005).

In conclusion, the ITS data proved a useful molecular tool to test the newly revised tribal limits

proposed for the Brassicaceae in Al-Shehbaz et al. (2006). In the present study, the ITS data supported the recognition of the tribes: Hesperideae (unigeneric, *Hesperis*) and Chorisporeae (3 genera, including *Chorisporea*, *Diptychocarpus*, and *Parrya*), whereas the Anchonieae and the Euclidieae each separated into two distinct and distant clades (designated here as Anchonieae I and II and Euclidieae I and II). The data also supported the exclusion of eight genera from the latter four tribes, with appropriate tribal assignment given in parentheses: *Aubrieta* (Arabideae), *Blennodia* (Camelineae), *Erysimum* (Camelineae), *Goldbachia* (unresolved), *Hesperidanthus* (Schizopetaleae), *Notothlaspi* (unresolved), *Pseudocamelina* (Thlaspidiae), and *Zuvanda* (unresolved).

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